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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Feeding - dog (82-1b)

SHAUGHNESSY NO./TOX. CHEM. NO.: 110003 / New Chemical

ACCESSION NO./MRID NO.: 434441-02

DP BARCODE/SUBMISSION NO.: D209722 / S477588

TEST MATERIAL: XDE-105

SYNONYMS: Spinosad (proposed common name for Factor A + Factor D)

LABORATORY PROJECT ID NUMBER: IET 91-0079

SPONSOR: DowElanco Division, Dow Chemical Japan Ltd., Seavans  
North, Tokyo, Japan

TESTING FACILITY: The Institute of Environmental Toxicology, 2-  
772, Suzuki-cho, Kodaira-shi, Tokyo 187,  
Japan

TITLE OF REPORT: XDE-105: 13-Week Oral Subchronic Toxicity  
Study in Dogs

AUTHOR(S): Takanori Harada

REPORT ISSUED: September 1, 1994

CONCLUSION: XDE-105 (Spinosad, 88.0% pure) was tested in a 13-week oral feeding study in male and female Beagle dogs. The chemical was mixed in the diet at the following dose levels: 0, 150, 300, 1350/900 ppm (males) or 900 ppm (females). These levels corresponded to 0, 4.89, 9.73 or 33.4 mg/kg/day for the low, mid- and high dose males and 0, 5.38, 10.47 or 29.9 mg/kg/day for the low, mid- and high dose females, respectively. The concentration of 1350 ppm was reduced from 1350 ppm to 900 ppm in males on day 38.

At 300 ppm and above, cytoplasmic vacuolation or vacuolated cell aggregation was observed in a variety of tissues in both sexes as well as atrophic gastric mucosa. At 1350/900 ppm, arteritis was observed in a variety of tissues in both sexes. In addition, Kupffer cell proliferation in the liver, atrophic white pulp in the spleen, focal necrosis/cellular depletion in the bone marrow and thymic atrophy were also observed. Clinical signs included periocular sebum, decreased spontaneous motor activity, unsteady standing posture and watery, red/black stools and/or loose

stools. One male was killed in extremis. Decreases in mean body weights (19% for males, 12% for females) and food consumption were observed in both sexes, particularly males. Evidence of anemia was found in the hematological examinations (decreases in hematocrit, hemoglobin and erythrocytes) as well as decreases in white blood cell counts, lymphocytes and reticulocytes. Decreases in albumin and A/G ratio and increases in globulin, total cholesterol, GOT, ALP and GPT were also observed. The increases in the latter three were slight, in only one sex and in one case due to only one dog. There were increases in several organ weights, although most in only one sex and/or in only absolute or relative weights. Increases in spleen and liver weights were supportive of the microscopic and/or clinical chemistry results. The NOEL is 150 ppm (4.89 (♂) or 5.38 (♀) mg/kg/day) and the LEL is 300 ppm (9.73 (♂) or 10.47 (♀) mg/kg/day) based on microscopic changes in a variety of tissues, clinical signs of toxicity, decreases in mean body weights and food consumption and biochemical evidence of anemia and possible liver damage.

Classification: Core Guideline

Testing Guideline Satisfied: 82-1(b)

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: XDE-105 (chemical name not available - huge molecule)

Description: Off-white to pale yellow powder

Lot #: AGR293707

Purity: 88.0%

Source: Not stated, assumed Sponsor

Vehicle: Test diet

Positive Control: N/A

2. Test Animals

Species and Strain (sexes): Male and female Beagle dogs

Age: 5-6 months at receipt; 6-7 months at initiation

Weight(s): 5.4 - 7.2 kg at receipt

Source(s): Ohito Biotech Center Inc. (Shuzenzi-cho, Tagatagun, Shizuoka)

3. Procedure

- a. Dietary Preparation: A specified amount of the test material was mixed with part of the basal diet in a mortar as a "pre-mixture". The pre-mixture was then blended with the remaining part of the basal diet with a mixer.

Frequency of preparation: Once prior to initiation of treatment and every 4 weeks thereafter.

Storage conditions: The prepared diets were placed in plastic bags, sealed, placed in plastic containers and stored in a dark and cold (about 4 °C) room until used. The prepared diet was then transferred into an aluminum container and retained in the animal room at room temperature until provided to the animals (up to 10 days).

Stability Analyses: Stability of the test substance in the diet was conducted on a sample dietary level of 200 ppm. The samples had been stored in a dark and cold room for 5 weeks with a subsequent 10-day storage at room temperature. Stability studies were also conducted on the moistened diet containing the test substance that had been stored at room temperature for 24 hours.

Homogeneity Analyses: Homogeneity of the test substance in the test diets was determined on samples taken from the top, middle and bottom of the mixer. These samples were taken from all dose groups on one date and for the high dose group on a second date.

Concentration Analyses: Concentration analyses for all dose groups were conducted on the same dates as the homogeneity analyses plus two additional dates. These analyses were conducted on the samples taken for the homogeneity analyses plus additional samples taken from the middle of the mixer. On the dates when no homogeneity analyses were conducted, the samples for the concentration analyses were taken from the middle of the mixer.

- b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of a 4-week range finding study in which dogs were fed XDE-105 at one of the following dose levels: 0, 200, 2000 or 4000 ppm (one dog/sex/dose). In that study, the dogs fed 4000 ppm were sacrificed in extremis due to deteriorated physical condition. At 2000 ppm, the following effects were observed in both dogs: "body weight loss, lower food consumption, anemia, various abnormalities in blood biochemical parameters and pathological changes in various organs". No effects were observed at 200 ppm.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered		Main Study	
	ppm	mg/kg/day	3 months male	female
Contr.	0	0	4	4
1	150	5	4	4
2	300	10	4	4
3	1350/900 <sup>a</sup>	45/30	4	
4	900	30		4

<sup>a</sup>The dietary concentration for the high dose males was reduced from 1350 to 900 ppm on day 38 because one male dog died from weakness caused by the test substance.

- d. Clinical Observations and Mortality: All animals were observed once daily for morbidity, mortality and clinical signs of toxicity. A careful physical examination was conducted once/week.
- e. Body Weight Determinations: Body weights were recorded at initiation of treatment, weekly during the treatment period and at termination.
- f. Food and/or Water Consumption: Food consumption was measured daily. Chemical intake was calculated from the body weight and food consumption data and the nominal dose level.
- g. Ophthalmological Examinations: These examinations were conducted prior to initiation and after 13 weeks of treatment. The following items were examined: eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body and fundus.
- h. Clinical Pathology: (\*) recommended by Guidelines
- 1) Hematology:
- Collection times for blood (including # of animals):  
Hematological examinations were conducted on all surviving animals prior to initiation and at weeks 7 and 13. Blood samples were withdrawn from the cephalic vein of each animal following overnight starvation.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB conc. (MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*	x	Reticulocytes
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X	Electrolytes:	X	Other:
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase	x	Triglycerides
x	Creatinine phosphokinase*	x	A/G Ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma-glutamyl transpeptidase (GGTP)		

3) Urinalysis:

Collection times for urine (including # of animals):

Urinalysis examinations were conducted prior to treatment and after 13 weeks of treatment. For each animal, urine was pooled for 24 hours.

The following CHECKED (X) parameters were examined:

X	Appearance*	X	Glucose*
x	Volume*	x	Ketones*
	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*	x	Urobilinogen

i. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: One animal was found moribund at week 5. This animal was anesthetized with pentobarbital sodium and then euthanized by exsanguination from the carotid artery. The animal was subjected to a complete autopsy that included the examinations for the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals were euthanized as above and subjected to gross examinations.

j. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: all animals.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: all animals.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (\*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		Glandular
x	Ileum*		Urogenital	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder	x	Mammary gland*
x	Rectum*	xx	Testes*	xx	Parathyroids*
xx	Liver*	x	Epididymides	xx	Thyroids*
x	Gall bladder*	x	Prostate		Other
xx	Pancreas*		Seminal vesicle	x	Bone*
	Respiratory	xx	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*			x	All gross lesions and masses
				x	Faucial tonsils

k. Statistical Analyses: According to the report, "the following statistical methods were used to determine the significance of the results:

Statistical methods	Data for analysis
Multiple comparison test: Dunnett's or Scheffe's method	Body weight Urine volume Urine specific gravity Hematology Blood biochemistry Organ weights
Mann-Whitney's U test	Food consumption Urinalysis (except urine volume and specific gravity)
Fisher's exact probability test	Clinical signs Mortality Ophthalmology Pathology

B. RESULTS:

1. Dietary Preparation: The stability of the test substance in the diet at 200 ppm for the samples that had been stored in a dark and cold room for 5 weeks with a subsequent 10-day storage at room temperature was 93% of the nominal value. The stability of the test substance in the moistened diet that had been stored at room temperature for 24 hours was 98% of the nominal value. The tables supporting these values were not provided in the report. For homogeneity of the test substance in the diet, the coefficient of variation for each dose level was within 1.1%. Tables were provided in the report to support this value. The mean concentrations of the test chemical in the diet at the nominal levels of 150, 300, 900 or 1350 ppm were 144, 290, 879 or 1335 ppm, respectively. The values were within 96-99% of the target concentrations. These values were supported by tables in the report.
2. Clinical Observations and Mortality: In the 1350/900 ppm group, clinical signs included periocular sebum, decreased spontaneous motor activity, unsteady standing posture and watery, red/black stools in males and loose stools in females. The authors of the report believed that the abnormalities in motor activity in the males was due to severe inanition due to markedly lower food consumption. One high dose male was found moribund and was killed in extremis at week 5 because of "unfavorable prognosis". The dog had exhibited marked body weight loss, lower food consumption, decreased spontaneous motor activity and unsteady standing posture. The report stated that no clinical signs or deaths were observed in any of the other treated groups. However, it is noted that vomit: foamy fluid was observed in at least one animal in all treated male groups and in all female groups, including controls. The following table summarizes the observed clinical signs of interest.

Clinical Observations													
Clinical sign	Dose (ppm)	0			150			300			1350/900		
	Treatment Weeks	1-4	5-8	9-13	1-4	5-8	9-13	1-4	5-8	9-13	1-4	5-8	9-13
Males													
	No. of animals examined	4	4	4	4	4	4	4	4	4	4	4*	3
Vomit: foamy fluid		0	0	0	1	1	1	2	1	2	2	2	1
Periocular region: sebum		0	0	0	0	0	0	0	0	0	1	1	2
Decreased spontaneous motor activity		0	0	0	0	0	0	0	0	0	1	2	0
Unsteady standing posture		0	0	0	0	0	0	0	0	0	1	1	0
Watery, black stools		0	0	0	0	0	0	0	0	0	0	1	0
Females													
	No. of animals examined	4	4	4	4	4	4	4	4	4	4	4	4
Loose stool		1	0	0	0	0	0	0	0	0	4	1	0
Vomit: foamy fluid		2	1	1	2	1	0	2	0	0	2	0	0
Vaginal bloody discharge		0	0	1	0	0	2	0	0	1	0	0	1

\*One animal died at week 5.

\*Number of animals

3. Body Weight Determinations: A decrease in mean body weight was observed in the high dose male group, beginning at approximately week 7 and lasting throughout the rest of the study. By the end of the study, high dose males weighed approximately 19% less than the control group. One of the three high dose males was within the control range, but the other two weighed significantly less than the control dogs. In females, this decrease was not as evident; the mean body weight of high dose females was approximately 88% of the control group by study termination. Two of the 4 females were within the range of the control animals and 2 were somewhat less than the control animals. The following table summarizes mean body weights for both sexes.

Mean Body Weights (kg)				
Dose Level (ppm) Weeks	0	150	300	1350/900 <sup>a</sup>
Males				
0	7.7 ± 0.7	7.7 ± 0.5	7.7 ± 0.6	7.8 ± 0.4
4	8.7 ± 0.8	8.8 ± 0.4	8.7 ± 0.6	8.3 ± 0.9
8	9.4 ± 0.7	9.6 ± 0.3	9.5 ± 0.5	8.3 ± 1.6 <sup>b</sup>
13	10.1 ± 0.7	10.3 ± 0.2	10.2 ± 0.5	8.2 ± 2.4 <sup>b</sup>
Females				
0	7.5 ± 1.1	7.4 ± 0.6	7.4 ± 0.7	7.4 ± 0.6
4	8.3 ± 1.2	8.1 ± 0.8	8.1 ± 0.8	8.2 ± 0.7
8	8.8 ± 1.4	8.4 ± 1.2	8.5 ± 1.0	8.1 ± 1.0
13	9.3 ± 1.7	8.7 ± 1.4	8.9 ± 1.0	8.2 ± 1.1

<sup>a</sup> 1350/900 in males only; 900 ppm in females throughout the entire study.

<sup>b</sup> Only three animals were available for measurement.

4. Food and/or Water Consumption: Food consumption appeared to be less than the control groups for both sexes at the high dose. The following table summarizes food consumption for both sexes.

Mean Food Consumption (g/dog/day) <sup>a</sup>				
Dose Level (ppm) Weeks	0	150	300	1350/900 <sup>b</sup>
Males				
1	300	300	282	300
4	300	300	300	235
8	300	300	300	261 <sup>c</sup>
13	300	300	300	229 <sup>c</sup>
Females				
1	300	300	288	300
4	300	300	293	294
8	300	300	293	249
13	298	300	300	252

<sup>a</sup> Calculated from the following formula: [feeding amount (300 g diet + 300g water) - food residue] / 2

<sup>b</sup> 1350/900 in males only; 900 ppm in females throughout the entire study.

<sup>c</sup> Only three animals were available for measurement.

The mean chemical intake values were calculated to be 0, 4.89, 9.73 or 33.4 mg/kg/day for the low, mid- and high dose males and 0, 5.38, 10.47 or 29.9 mg/kg/day for the low, mid- and high dose females, respectively.

5. Ophthalmological Examinations: No treatment-related effects were observed.

6. Hematology: In the high dose group of both sexes, significant decreases in hematocrit and hemoglobin were observed. In males, these were accompanied by decreases in red blood cells, lymphocytes and reticulocytes. White blood cell counts and platelets were decreased also, although not statistically significantly so. In females, there was a significant decrease in mean corpuscular hemoglobin. Reticulocytes were increased and erythrocytes, white blood cells, lymphocytes and platelets were decreased, although not statistically significantly so. The above observations indicate possible anemia. The following table summarizes selected results from male and female dogs at study initiation and after 13 weeks.

Selected Group Mean Hematology Data at 0 and 13 Weeks									
Dose (ppm)	# Animals	Ht (%)	Hb (g/dl)	RBC ( $10^6/\text{mm}^3$ )	WBC	Lymph	PLT ( $10^3/\text{mm}^3$ )	Retics. (/10 <sup>3</sup> RBC)	MCH (pg)
Males									
Week 0									
0	4	38.3	13.2	5.89	10.8	4.5	292	-	22.4
150	4	36.4	12.6	5.53	10.9	5.5	330	-	22.7
300	4	38.0	13.1	5.89	11.7	4.3	349	-	22.3
1350/ 900	4	34.8	12.1	5.32	9.6	3.7	316	-	22.8
Week 13									
0	4	43.0	14.7	6.49	10.9	4.5	307	13 ± 5	22.7
150	4	41.6	14.2	6.26	11.4	4.0	302	7 ± 3	22.7
300	4	43.0	14.7	6.63	9.2	3.7	300	8 ± 5	22.2
1350/ 900	3	31.4**	10.6**	5.12**	8.4	2.0*	279	2 ± 2*	20.8
Females									
Week 0									
0	4	40.5	14.1	6.26	10.4	4.9	373	-	22.5
150	4	42.0	14.3	6.36	9.8	3.5	335	-	22.5
300	4	41.7	14.2	6.28	9.5	3.8	343	-	22.7
900	4	43.7	15.0	6.65	8.9	3.4	322	-	22.5
Week 13									
0	4	45.2	15.5	6.81	11.8	4.1	383	8 ± 3	22.8
150	4	47.6	16.1	7.13	10.6	3.5	332	12 ± 4	22.7
300	4	46.1	15.8	6.84	7.8	3.1	292	10 ± 5	23.0
900	4	40.2*	13.4*	6.20	7.3	2.6	282	20 ± 14	21.6**

\*Statistically significant ( $p < 0.05$ ); \*\*Statistically significant ( $p < 0.01$ )

7. Clinical Chemistry: In the high dose groups, statistically significant decreases in albumin and A/G ratio were observed in both sexes. Significant increases in globulin ( $\sigma$ ), total cholesterol ( $\sigma$ ), TG ( $\sigma$ ) and GOT ( $\sigma$ ) were also observed. In addition, non-statistically significant increases in alkaline phosphatase were observed in both sexes. This increase in males was probably not biologically significant. In females, the ALP value in 1/4 dogs was close to the control range and elevated in 3/4 dogs. GPT was also elevated in high dose females, although all of this elevation was due to one dog. This dog had normal values at initiation of treatment. The following table summarizes selected clinical chemistry values for male and female dogs at initiation of treatment and at study termination.

Selected Group Clinical Chemistry Data at 0 and 13 Weeks									
Dose (ppm)	# Animals Examined	Alb (g/dl)	Glob (g/dl)	A/G ratio	T. Chol (mg/dl)	TG (mg/dl)	ALP (U/l)	GOT (U/l)	GPT (U/l)
Males									
0 Weeks									
0	4	2.94	2.51	1.19	133	38	102	31	41
150	4	3.05	2.51	1.22	127	39	128	32	36
300	4	2.98	2.78	1.09	136	34	90	29	29
900/1350	4	2.94	2.29	1.29	112	31	97	29	39
13 Weeks									
0	4	3.21	2.55	1.26	128	39	69	35	47
150	4	3.18	2.62	1.22	129	38	75	37	45
300	4	3.30	2.84	1.19	147	37	65	33	46
900/1350	3	2.49**	3.65**	0.68**	162*	52*	93	67	70
Females									
0 Weeks									
0	4	3.05	2.27	1.35	116	32	111	33	38
150	4	2.98	2.40	1.25	114	35	95	32	35
300	4	3.14	2.45	1.30	135	44	113	33	37
900	4	3.17	2.29	1.39	130	40	112	30	49
13 Weeks									
0	4	3.35	2.56	1.32	124	46	61	34	44
150	4	3.28	2.76	1.20	136	44	62	32	36
300	4	3.30	2.63	1.29	156	45	78	37	39
900	4	2.60**	3.19	0.83**	145	59	161 ± 143	172 ± 227*	451 ± 789

\*Significantly different from control group (p<0.05); \*\*Significantly different from control group (p<0.01)

8. Urinalysis: In high dose females, there appeared to be a slight decrease in urinary pH at study termination. No other abnormalities were observed. The following table summarizes the results.

pH Values at 0 and 13 Weeks									
			pH						
Week	Dose (ppm)	# Animals Examined	5.0	6.0	6.5	7.0	7.5	8.0	8.5
Males									
0	0	4				1 <sup>a</sup>	1	1	1
	150	4			1	1			2
	300	4			1				3
	1350/ 900	4					1		3
13	0	4						1	3
	150	4							4
	300	4							4
	1350/ 900	3			1		1		1
Females									
0	0	4						1	3
	150	4						2	2
	300	4					1	1	2
	900	4						1	3
13	0	4							4
	150	4						1	3
	300	4				1	1		2
	900	4			1	1	1	1*	

<sup>a</sup>Number of animals

\*Significantly different from control at 5% level of probability.

9. Gross Pathology: Most macroscopic lesions were observed in high dose animals and at no other dose level, including controls in either sex.

Males:

The following were seen at the high dose level in one animal each: emaciation, atrophy of the thymus, enlargement of the lymph nodes, yellow and brown spots on the lungs, black sandy contents of the gall bladder, edema and brownish color in the pancreas, enlargement of the kidneys (considered to be congenital), red spot on the thyroid, sebum in the eye and hematoma-like mass in the thoracic cavity.

The following were seen at the high dose level in two animals each: whitish granular mucosa of the stomach, distended stomach with diet and pale liver and kidneys.

Females:

The following were observed at the high dose level in one animal each: enlargement of the spleen, distension of the stomach with diet, mud-like contents in the large intestine, pale and/or enlarged liver and black sandy contents in the gallbladder.

Whitish granular mucosa/whitish mucosa of the stomach was observed in 3 high dose animals.

10. Organ Weights: Statistically significant increases in absolute and relative pancreatic weights were observed in high dose males. Increases were also observed in high dose females, although not statistically significantly so. Absolute and relative spleen weights were increased in both sexes at the high dose. These increases were not statistically significant. Liver weights were also slightly increased in both sexes, although only relative liver weights in high dose females were statistically significant. Thyroid weights were increased in high dose males; relative thyroid weights were statistically significantly increased. The following table summarizes selected results.

Selected Mean Absolute Organ Weights After 13 Weeks of Treatment							
Dose (ppm)	# Dogs	Thyroid (mg)	Heart (g)	Pancr. (g)	Liver (g)	Kidneys (g)	Spleen (g)
Males							
0	4	701	70.4	13.8	255	40.0	19.2
150	4	860	72.5	20.1 <sup>a</sup>	253	41.4	19.1
300	4	875	80.1	17.7	269	43.7	21.0
1350/900	3	1045	72.3	20.2 <sup>a</sup>	327	46.3	27.4
Females							
0	4	707	68.3	15.5	236	35.6	19.2
150	4	1020	68.5	19.4	224	36.0	20.3
300	4	798	66.4	17.3	236	36.6	20.6
900	4	844	65.4	21.5	281	39.8	30.3

Selected Mean Relative Organ Weights After 13 Weeks of Treatment							
Dose (ppm)	# Dogs	Thyroid	Heart	Pancr.	Liver	Kidneys	Spleen
Males							
0	4	0.0070	0.70	0.14	2.53	0.40	0.19
150	4	0.0084	0.71	0.20	2.47	0.40	0.19
300	4	0.0086	0.79	0.18	2.64	0.43	0.21
1350/900	3	0.0127 <sup>b</sup>	0.90 <sup>b</sup>	0.25 <sup>a</sup>	4.05	0.58	0.35
Females							
0	4	0.0077	0.74	0.17	2.56	0.39	0.22
150	4	0.0118 <sup>b</sup>	0.80	0.22	2.59	0.42	0.24
300	4	0.0091	0.75	0.20	2.68	0.41	0.24
900	4	0.0104 <sup>a</sup>	0.80	0.27	3.49 <sup>a</sup>	0.49	0.38

<sup>a</sup>Significant (p<0.05); <sup>b</sup>Significant (p<0.01)

11. Histopathology: The following table summarizes selected microscopic lesions that were observed either at study termination or in the 1 dog that was sacrificed in extremis. In high dose animals, cytoplasmic or vacuolated cell aggregation was observed in both sexes in a variety of tissues. Arteritis was also observed in a variety of tissues. The report stated that "nervous tissues immediately adjacent to the affected blood vessels demonstrated vacuolation consistent with focal edema secondary to the vascular inflammation. There was no evidence of nervous degeneration/necrosis, reactive gliosis or neuronophagia in these lesions." In the high dose group, atrophic gastric mucosa, Kupffer cell proliferation in the liver, atrophic white pulp in the spleen, focal necrosis/cellular depletion in the bone marrow and thymic atrophy were also observed. An aneurysm in the mediastinal region of the thoracic cavity was found in the animal that died prior to termination. In the mid-dose group, cytoplasmic vacuolation or vacuolated cell aggregation was also observed in a variety of tissues. In addition, atrophic gastric mucosa was found in 2 females.

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900 <sup>a</sup>
Males					
Pericarditis of heart		1/4	0/4	0/4	2/4
Atrophy of thymus		0/4	0/4	0/4	2/4
Vacuolated cell aggregation in white pulp of spleen		0/4	0/4	1/4	4/4 <sup>b</sup>
Atrophic white pulp of spleen		0/4	0/4	0/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of cervical lymph nodes		0/4	0/4	0/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of mesenteric lymph nodes		0/4	0/4	1/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of faucial tonsil		0/4	0/4	2/4	4/4 <sup>b</sup>
Foamy cell aggregation of the lung		0/4	0/4	0/4	4/4 <sup>b</sup>
Arteritis of the lung		0/4	0/4	0/4	1/4
Atrophic mucosa of the stomach		0/4	0/4	0/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of the ileum		0/4	0/4	1/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of the cecum		0/4	0/4	0/4	3/4

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900 <sup>a</sup>
Vacuolated cell aggregation in lymph follicles of the colon		0/4	0/4	2/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of the rectum		0/4	0/4	2/4	3/4
Vacuolated hepatocytes		0/4	0/4	0/4	3/4
Kupffer cell proliferation		0/4	0/4	0/4	3/4
Vacuolated acinar cells of the pancreas		0/4	0/4	2/4	4/4 <sup>b</sup>
Testis:					
Spermatid giant cells		0/4	0/4	1/4	2/4
Arteritis		0/4	0/4	0/4	2/4
Vacuolated seminiferous epithelial cells		0/4	0/4	0/4	3/4
Decreased spermatogenesis		0/4	0/4	0/4	1/4
Arteritis of the epididymis		0/4	0/4	0/4	2/4
Vacuolated glandular cells of the parathyroid		0/4	0/4	0/4	4/4 <sup>b</sup>
Brain (cerebrum) - arteritis in the meninx		0/4	0/4	0/4	1/4
Vacuolated nerve cells in the brain		0/4	0/4	0/4	3/4
Vacuolated nerve cells in the cervical spinal cord		0/4	0/4	0/4	4/4 <sup>b</sup>
Spinal cord (thoracic)					
Vacuolated nerve cells		0/4	0/4	0/4	2/4
Arteritis in nerve root/meninx		0/4	0/4	0/4	2/4

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900 <sup>a</sup>
Vacuolated nerve cells in the lumbar spinal cord		0/4	0/4	0/4	3/4
Arteritis in optic nerve		0/4	0/4	0/4	2/4
Thoracic cavity: Arteritis/ruptured aneurysm		-	-	-	2
Pleuritis		-	-	-	1

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900 <sup>a</sup>
<b>Females</b>					
Focal necrosis/cellular depletion of sternal bone marrow		0/4	0/4	0/4	2/4
Focal necrosis/cellular depletion of femoral bone marrow		0/4	0/4	0/4	3/4
Vacuolated cell aggregation in white pulp of spleen		0/4	0/4	1/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of cervical lymph nodes		0/4	0/4	2/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of mesenteric lymph nodes		0/4	0/4	2/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of faucial tonsils		0/4	0/4	3/4	4/4 <sup>b</sup>
Foamy cell aggregation of lungs		0/4	0/4	1/4	4/4 <sup>b</sup>
Atrophic mucosa of stomach		0/4	0/4	2/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of ileum		0/4	0/4	3/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of cecum		0/4	0/4	2/4	4/4 <sup>b</sup>
Vacuolated cell aggregation in lymph follicles of colon		0/4	0/4	1/4	4/4 <sup>b</sup>

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900 <sup>a</sup>
Vacuolated cell aggregation in lymph follicles of rectum		0/4	0/4	0/4	4/4 <sup>b</sup>
Liver: Vacuolated hepatocytes		0/4	0/4	0/4	1/4
Kupffer cell proliferation		0/4	0/4	0/4	3/4
Vacuolated acinar cells of pancreas		0/4	0/4	0/4	3/4
Vacuolated C-cells of thyroid		0/4	0/4	0/4	3/4
Vacuolated glandular cells of parathyroid		0/4	0/4	0/4	4/4 <sup>b</sup>
Vacuolated cortical cells of adrenal		1/4	1/4	1/4	2/4
Vacuolated nerve cells of cerebellum		0/4	0/4	0/4	1/4
Vacuolated nerve cells of pons		0/4	0/4	0/4	1/4
Vacuolated nerve cells of thoracic spinal cord		0/4	0/4	0/4	2/4
Vacuolated nerve cells of lumbar spinal cord		0/4	0/4	0/4	3/4
Thoracic cavity: arteritis		-	-	-	1

<sup>a</sup>1350/900 ppm in males dogs; only 900 ppm in female dogs.

<sup>b</sup>Significant at  $p < 0.05$ .

() = Number of tissues examined.

12. Quality Assurance Measures: Signed Good Laboratory Practice and Quality Assurance Statements were provided.

C. DISCUSSION: This was a well conducted study. The study is classified as Core Guideline and is acceptable for regulatory purposes. The NOEL is the lowest dose tested (150 ppm or 4.89 mg/kg/day for males and 5.38 mg/kg/day for females). The LEL is the mid-dose (300 ppm or 9.73 mg/kg/day for males and 10.47 mg/kg/day for females). Clinical signs of toxicity were observed in the high dose group. These included some neurological signs (decreased spontaneous motor activity and unsteady standing posture) which the authors stated were due to severe inanition. Decrease in body weights were especially observed in high dose males along with decreased food consumption in both sexes at the high dose. The hematological examinations indicated evidence of anemia and inanition. The clinical chemistry examinations indicated some evidence of liver injury, although slight. The microscopic examinations indicated chemically-related effects in a variety of tissues. Generally, these effects involved cytoplasmic or vacuolated cell aggregation and arteritis in a variety of tissues. In addition, atrophic gastric mucosa, Kupffer cell proliferation in the liver, atrophic white pulp in the spleen, focal necrosis/cellular depletion in the bone marrow and thymic atrophy were also observed.